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HOMOLOGOUS STRUCTURES OF NUCLEAR AND GTPase-LINKED PLASMA MEMBRANE RECEPTORS SUGGEST ANALOGOUS MECHANISMS OF ACTION

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The molecular structures of nuclear and GTPase-linked plasma membrane receptors are compared here in the light of a recent finding suggesting that histone H1 may be an ATP/GTPase involved in transduction of the action of nuclear receptors. Considerable homology and conservation of the regions responsible for the interaction of the plasma membrane receptors with GTPases was observed in the nuclear receptors, thus suggesting analogous mechanisms of action and a common evolutionary origin for the two receptor families.

A large family of structurally homologous receptors for extracellular ligands reside in the plasma membrane and act by promoting GDP/GTP exchange in a variety of GTP-binding proteins (1-3). These GTPases, such as transducin ($G_{\alpha T}$) and various subtypes of $G_{\alpha S}$ and $G_{\alpha i}$, are involved in receptor-effector coupling of the ligand-induced signals, being capable, upon the binding of GTP, of interacting with effector proteins such as adenylate cyclase, cGMP-specific phosphodiesterase and phospholipase-C. Similarly, GAP may be regarded as an effector for ras-p21 (4,5), this being one of the GTPases whose mechanism of action has been studied in most detail so far (6-9). The effector proteins subsequently catalyze the synthesis of a variety of second messengers.

In general, nuclear receptors exert their effects through binding to DNA (10-12). A ligand such as steroid hormone, T_3, retinoic acid or 1,25-dihydroxycholecalciferol is needed for this interaction. The ligand enables dissociation of the receptor from depository hsp90 (13) and dimerization (14). Upon binding to DNA, nuclear receptors are thought to bring about a change in the chromatin structure that results in induction or repression of certain genes (10,15-18). The mechanisms by which the induction is brought about are unknown, although they may involve disruption (15) or a more subtle structural change in a specifically positioned nucleosome in the promoter region of the inducible gene (16-18). These changes may be accompanied by changes in histone acetylation (19).

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Histone H1 is a eukaryotic repressor capable of binding nucleotides such as GDP, ADP, GTP and ATP (20), which modulate its binding to DNA (21). H1 is thought to be less loosely bound to chromatin in active areas as compared with the areas not as efficiently expressed (22,23), although it is still present (24). We have recently put forward a hypothesis that H1 may be an ATP/GTPase involved in transduction of the action of nuclear receptors (25), the mechanism of action of which may thus be regarded as similar to that of the GTPase-linked plasma membrane receptors. It may thus be assumed that upon binding to DNA and chromatin activation nuclear receptors cause the dissociation of H1 from the nucleosome hinge/linker region at least part of their action. In order to lend further support to this hypothesis, the known molecular structures of these two receptor families are compared here. The results indeed suggest that nuclear and GTPase-linked plasma membrane receptors may act through analogous mechanisms.

METHODS

The computer programs described by Pustell and Kafatos (26-28) were used for protein sequence analysis, and the Genebank^{R} Genetic Sequence Data Bank to search for various sequences.

RESULTS AND DISCUSSION

A considerable degree of identity is demonstrable at the level of their primary structures between the conserved DNA/ligand-binding domain of nuclear receptors and a C-terminal region of GTPase-linked plasma membrane receptors, the region responsible for GTPase coupling (Fig. 1A). Alignment of a putative rat dopamine receptor (D_2R) (29) and the human glucocorticoid receptor (30) is shown in Fig. 2. These two display one of the highest degrees of homology identified so far, 26 % identity and 38 % similarity being calculated between the 150-amino acid regions of homology. These regions of D_2R and GR are also likely to fold to similar higher order structures, as demonstrable by Chou-Fasman analysis and their highly similar hydropathy plots (Fig. 1B). The hydropathy plots differ significantly only at the C-terminal ends of the transmembrane domains of the plasma membrane receptors (see below), these regions being more hydrophilic in the nuclear receptors.

How do the present findings relate to what is known at present of the structure and function of various domains of D_2R and GR? The polypeptide chains of all GTPase-linked plasma membrane receptors comprise seven hydrophobic transmembrane domains (TM1 to TM7) responsible for anchoring the receptor molecules to the membrane, and correspondingly four extracellular (EC1 to EC4) and four intracellular domains (IC1 to IC4)

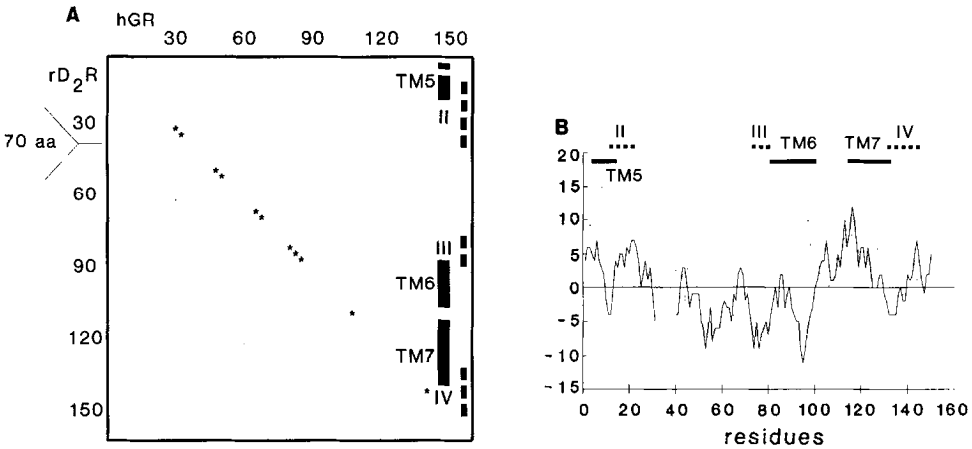

Fig. 1. Panel A shows the homology hash matrix of human glucocorticoid receptor (GR; residues 434 to 594) and a putative rat dopamine receptor (D_2R; residues 195 to 415). A part of the variable third intracellular domain (IC3) of the plasma membrane receptor has been omitted in the case of D_2R, since no corresponding region can be identified in GR. Panel B shows hydropathy plots of the same region in GR (—) and D_2R (- -). Black bars depict the locations of transmembrane (TM) domains TM5, TM6 and TM7 in D_2R, and dotted lines the regions II, III and IV responsible for interaction with a GTPase, as depicted in Fig. 3.

between them (Fig. 3) (31). Four regions in IC2, IC3 and IC4, numbered here I to IV, have been demonstrated by deletion and point mutagenesis to be responsible for the interaction of a ß-adrenergic receptor (ßAR) with the appropriate GTPase (32,33).

No similar structure can be deduced for nuclear receptors such as GR based on the existing data, although thorough functional deletion and

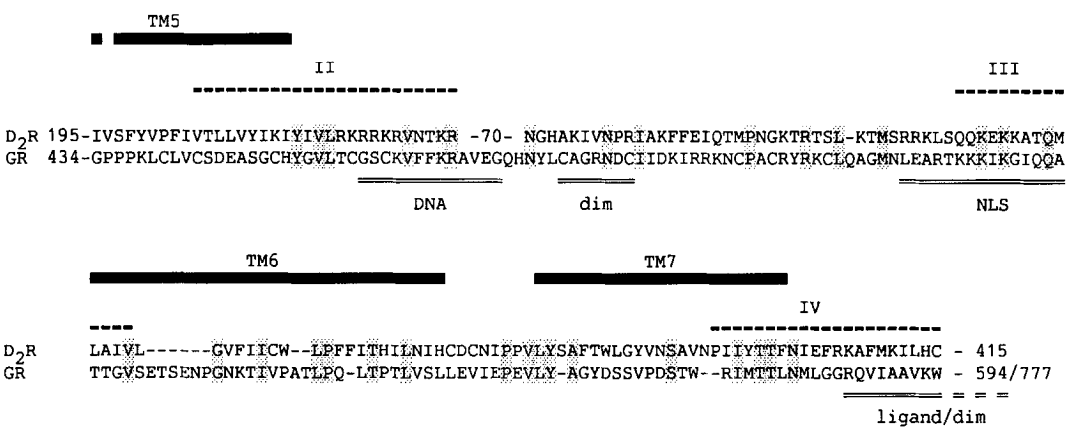

Fig. 2. Alignment of D_2R and GR. Identical amino acid residues are hatched, and gaps are introduced for maximal alignment. Black bars indicate the locations of TM5, TM6 and TM7 in D_2R, and dotted lines the regions II, III and IV responsible for interaction with a G protein in the case of ß-adrenergic receptors (ßAR). Double lines depict the functional domains of GR.

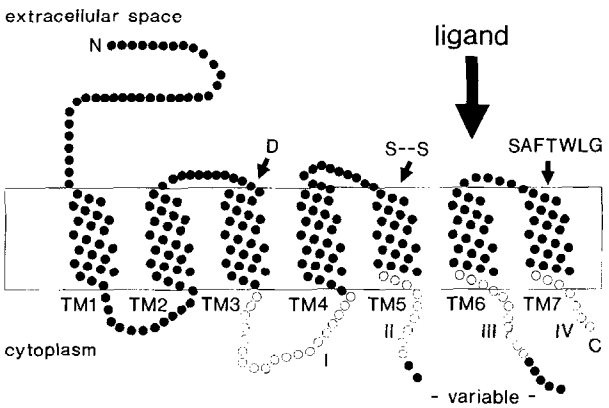

Fig. 3. Topological structure of GTPase-linked plasma membrane receptors such as βAR and D_2R. The figure has been modified from O'Dowd et al. (29). Seven transmembrane domains are designated TM1 to TM7 and four regions shown to be involved in receptor-G protein interaction numbered I to IV. The residues of TM3, TM5 and TM7 depicted here interact with an agonist ligand.

point mutation analyses have been performed to locate the domains responsible for the diverse functions of the polypeptide chain (see 10-12), and a tertiary structure has been reported for the well-conserved DNA-binding domain of GR (34,35). Interestingly, regions II, III and IV involved in the receptor-GTPase coupling are the best conserved areas between D_2R and GR, displaying 30 %, 33 % and 21 % identity respectively. These three regions are also well conserved within the nuclear receptor family. No counterpart can be identified in GR for region I of βAR.

Region II of D_2R corresponds to the first Zn^{2+} finger of GR (34), the function of which is unknown. Some subtypes of nuclear receptors that are lacking the first Zn^{2+} finger have also been identified recently (36). The extreme N-terminal part of IC3 corresponds to the DNA binding α-helix of the nuclear receptors (11,12,34), and this region is not particularly well conserved between or within the two receptor families. The variable region in IC3 corresponds to a variable region in the nuclear receptors between the DNA binding α-helix and the second Zn^{2+} finger. Consistent with this, the two latter sequences are coded by two separate exons in a number of nuclear receptors, the variable region matching the exon-exon boundary (37). The second Zn^{2+} finger is evidently responsible in part for the dimerization of GR (34).

In addition to a nuclear location signal (see 10-12), the counterpart of region III harbors a region which is partly responsible for the activation of transcription by GR. Both these regions may thus be responsible for the activation of a GTPase by GR. Consistent with the rationale that the functionally important regions are conserved between

D_2R and GR, a highly variable region of nuclear receptors residing between the DNA and ligand-binding domains corresponds to EC4 in plasma membrane receptors. This variable region is thought to form a hinge between the DNA and ligand binding domains in GR.

Region IV of D_2R and its counterpart in GR are flanked by ligand binding domains in both receptor families (14,32). There is no evidence that this region is responsible for any particular function in the case of nuclear receptors, but the region residing towards the C terminus is responsible for recognition of the ligand by nuclear receptors (14), corresponding to the highly variable IC4 of plasma membrane receptors, while in the case of the latter receptor family recognition of the ligand takes place primarily through TM3, TM5 and TM7 (33).

In conclusion, a considerable degree of homology was demonstrated here between a nuclear receptor and the domain responsible for the interaction of a plasma membrane receptor with GTPases. The present results suggest thus that the nuclear and GTPase-linked plasma membrane receptors may share a common evolutionary origin, and that they may function through analogous mechanisms, i.e. promoting nucleotide exchange in an appropriate ATP/GTPase. Nuclear receptors may thus act by promoting selective dissociation of H1 from chromatin. Specific ligands supposedly activate these two receptor families through different mechanisms. The ligands enable specific dimerization of nuclear receptors through a C-terminal region and thereby target the receptor molecules to appropriate sites on DNA, while the ligand-binding domain of GTPase-linked plasma membrane receptors is buried within the present homologous region and a conformational change is thought to be responsible for the activation of the receptor.

We have recently obtained experimental evidence to support the present hypothesis. Synthetic peptides of both D_2R and GR corresponding to one of the regions thought to be responsible for the GTPase coupling promote the removal of GDP-Mg^{2+} from H1 and enable GDP/GTP exchange (N. Yli-Mäyry, T. Tarkka, R.-M. Mannermaa and J. Oikarinen, unpublished results).

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